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A number of circulating markers have been identified that have the potential to be used in the detection or prognosis of breast cancer. Unfortunately, no single marker is consistently increased in breast cancer patients when compared with the general population. We hypothesized, however, that a sophisticated analysis of large number of circulating markers would accurately detect breast cancer as well as provide a valuable tool for prognosis. Therefore, we proposed to develop a rapid and simple system to measure a large number of blood markers associated with breast cancer. We will accomplish this by developing an antibody microarray with antibodies specific to different blood markers. Currently, we have screened fourteen markers, and generated standard curves for eight of these markers. We have refined the microarray to measure markers with a sensitivity down to 0.5 pg/ml. This sensitivity will allow the use of this microarray to screen up to 200 serum samples from breast cancer patients and control patients. These data will be analyzed using sophisticated computer programs that are designed to find relationships in a complex data set such as this. These studies will result in a prototype chip that can be used for the rapid determination of circulating markers associated with breast cancer.

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Introduction

Circulating blood carries chemical information from every cell in the body in the form of proteins, hormones and other factors that can potentially be assayed to screen for cancers and other diseases. In the case of breast cancer, a number of circulating markers have been identified that have the potential to be used in the detection or prognosis of the disease. Unfortunately, no single marker is consistently increased in breast cancer patients when compared with the general population. We hypothesize, however, that a sophisticated analysis of a large number of circulating markers would accurately detect breast cancer as well as provide a valuable tool for prognosis. Therefore, we propose to develop a rapid and simple system to measure a large number of blood markers associated with breast cancer. We will accomplish this by developing an antibody microarray with antibodies specific to different blood markers and use this microarray to screen up to 200 serum samples from breast cancer patients and control patients. These data will then be analyzed using sophisticated computer programs that are designed to find relationships in a complex data set such as this. Once completed, these studies will result in a prototype chip that can be used for the rapid determination of circulating markers associated with breast cancer. This basic technology is likely to lead to the development of more advanced chips with wide application in screening, diagnosis, and prognosis of patients with breast cancer.

Body

In this year we have made significant progress toward accomplishing Task #1 (reprinted below from our approved Statement of Work).

Task 1. Design and test a diagnostic protein chip containing a repertoire (up to 25) of monoclonal antibodies specific to serum tumor markers associated with breast cancer (months 1-24).

- Develop a microarray chip containing up to 25 different antibodies that recognize circulating markers associated with breast cancer.
- Collect a preliminary number of serum samples from individuals that are apparently cancer-free and those with breast cancer. We estimate that we will have about 30-50 samples of each type by this time. These samples will be screened by Western blot methods to identify samples which have high and low levels of each targeted marker.

- Test the microarray chip using the sera identified in the above step. This will allow us to determine appropriate conditions for detection. Factors that potentially may be varied are amounts of antibodies used, either for binding to the spot or for detection; dilution of serum; incubation time; and source of antibody (some antibodies may not work satisfactorily).
- Day to day reproducibility and stability of the chips will also be determined.

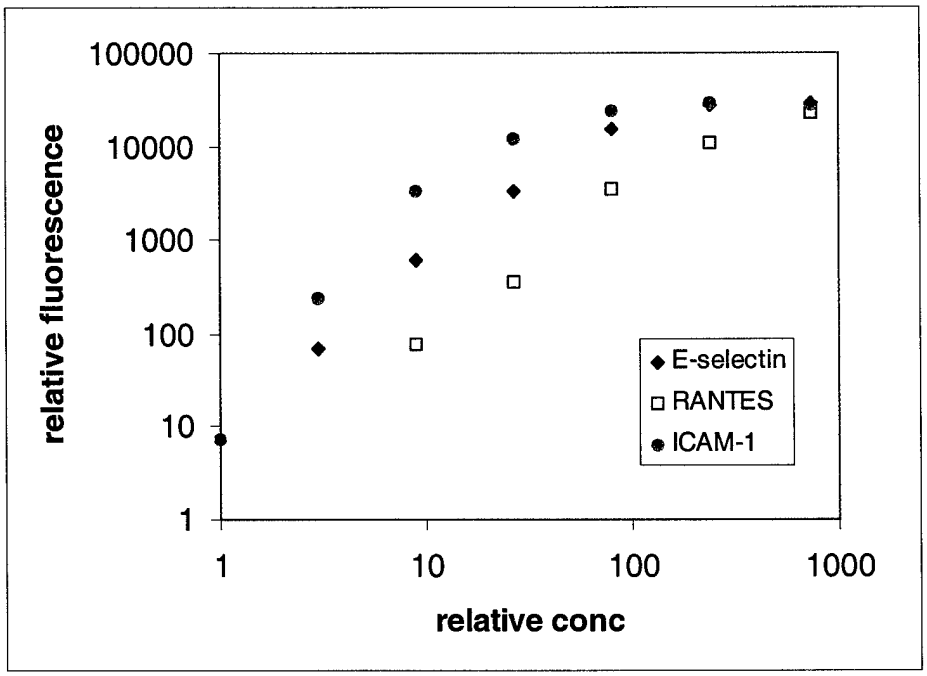
During this year we refined the microarray format using hepatocyte growth factor (HGF) as a test protein for detection. The microarray can detect HGF at sub-pg/ml concentrations in sample volumes of 100 microliters or less. Additionally, we showed that the microassay is quantitative and used the microassay to detect elevated HGF levels in sera from recurrent breast cancer patients. This work was published in the Journal of Proteomee Research and is included here as Appendix 1. We additionally show in this paper that multiple biomarkers can be simultaneously measured on a single microarray (Figure 5 of Appendix 1).

Currently we have the acquired antibodies and antigens to quantitatively measure the levels of 15 breast cancer biomarkers: CA15-3, cathepsin D, carcinoembronic antigen (CEA), EGF-R, E-Selectin, Fas-ligand, basic fibroblast growth factor (bFGF), HGF, I-CAM-1, matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), osteopontin, prostrate specific antigen (PSA), RANTES, vascular endothelial growth factor (VEGF). Of these we have generated standard curves for CA15-3, E-Selectin, Fas-ligand, HGF, I-CAM-1, PSA, RANTES, and VEGF (Figures 1 and Appendix 1). The quantitative range for all of these markers is between 2 to 3 orders of magnitude. Furthermore we are able to quantitate these markers within the expected physiological range for each marker. We are in the process of getting standard curves for EGF-R, MMP2 and MMP9. We have experienced some trouble with antibody reagents to the HER2, FGF and CEA biomarkers. We are currently experimenting with new antibody combinations for these biomarkers and have recently received reagents to set up assays to measure cathepsin D and osteopontin.

Figure 1. Standard curves for (A) E-selectin, RANTES and ICAM-1 and (B)

VEGF, FAS-ligand, CA15-3 and PSA.

A



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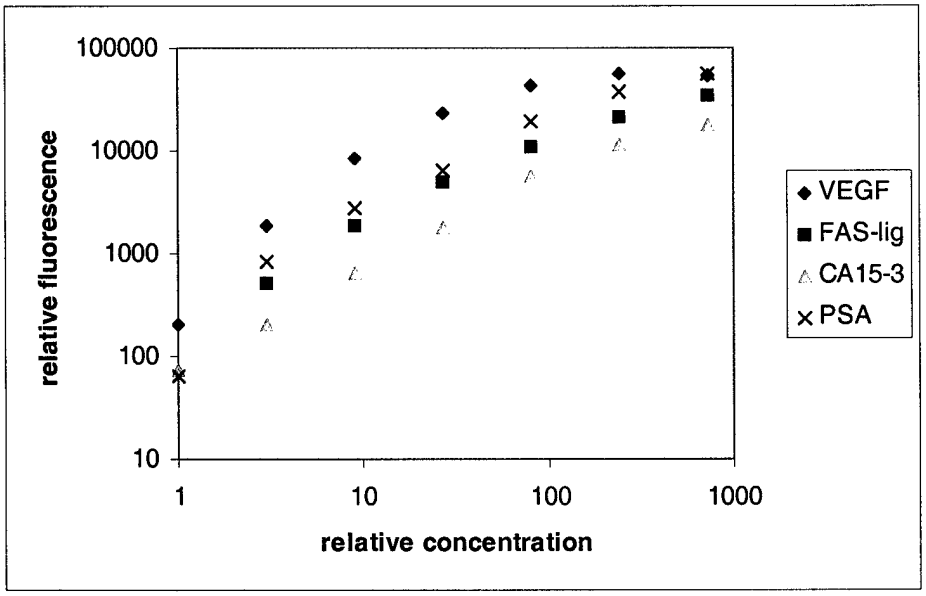

We have just begun to address Task #2 (reprinted here from our approved Statement of Work)

Task 2: Analyze approximately 100 serum samples from breast cancer patients and 100 from apparently healthy individuals for levels of the marker proteins. This data will then be analyzed using conventional statistics and bioinformatics software (SPIRE) developed at this institute to delineate associations between circulating markers and the presence and stage of breast cancer (months 25-36).

- The 200 serum samples will be analyzed using the microarray mAb chip developed in task 1.
- The data will be analyzed using the SPIRE software and conventional statistics.
- The resulting data will be used to evaluate the usefulness of the chip in the detection and prognosis of breast cancer as well as determining the contribution of individual markers to assessing breast cancer.

We have ordered a total of 200 serum samples; 50 normal controls, 50 samples from high risk women, 50 samples from women diagnosed with stage I or stage II breast cancer and 50 samples from women with recurrent breast cancer. We anticipate having a microarray set up to measure the 15 biomarkers listed above in a short time. When that is achieved it will take only a short time to simultaneously quantitate the levels of the biomarkers in each of these serum samples.

Key Research Accomplishments

- Further refinement of protein microarray resulting in a sensitive, quantitative, and reproducible assay.
- Demonstration of the utility of the microarray by comparing the concentration of serum HGF in woman with breast cancer and a healthy control group.
- Demonstated the ability to use the microarray for the simultaneous quantitation of multiple biomarkers.
- Standard curves for eight biomarkers generated.

Reportable Outcomes

- Woodbury RL, Varnum SM, Zangar RC. Elevated HGF Levels in Sera from Breast Cancer Patients Detected Using a Protein Microarray ELISA. 2002. Journal of Proteome Research, 1, 233-237.
- Woodbury RL, Varnum SM, Zangar RC. Elevated HGF Levels in Sera from Breast Cancer Patients Detected Using a Protein Microarray ELISA. Second Annual International Conference on Protein Microarrays, March 18-19, 2002.

Conclusions

We have made significant progress this year toward generating an antibody microarray for the quantitation of breast cancer biomarkers in serum. In a short time we will have assembled a complete repertoire of antibodies on an array and will begin to assay for the levels of cancer markers in serum samples. This type of antibody microarray has great potential for the rapid determination of circulating markers associated with breast cancer. This basic technology is likely to lead to the development of more advanced chips with wide application in screening, diagnosis and prognosis of patients with breast cancer.

References

Appendices

Appendix 1 is an original copy of our journal article referenced in the text.

Personel that have worked on this project over the last year are Drs. Richard C. Zangar, Susan M. Varnum and Ron Woodbury.

Elevated HGF Levels in Sera from Breast Cancer Patients Detected Using a Protein Microarray ELISA

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Elevated HGF Levels in Sera from Breast Cancer Patients Detected Using a Protein Microarray ELISA

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We developed an ELISA in high-density microarray format to detect hepatocyte growth factor (HGF) in human serum. The microassay can detect HGF at sub-pg/mL concentrations in sample volumes of 100 μL or less. The microassay is also quantitative and was used to detect elevated HGF levels in sera from recurrent breast cancer patients. The microarray format provides the potential for high-throughput quantitation of multiple biomarkers in parallel, as demonstrated with a multiplex analysis of five biomarker proteins.

Keywords: microarray • ELISA • breast cancer • hepatocyte growth factor

Introduction

Enzyme-linked immunosorbent assay (ELISA)-based immunoassays have been the mainstay of the clinical laboratory for decades; however, problems arise when limited sample volume is available and high-throughput analysis of multiple markers is required. Protein microarrays potentially permit the simultaneous measurement of many proteins in a small sample volume and therefore provide an attractive alternative approach for the quantitative measurement of proteins in serum. To develop this potential, it is necessary that protein microarrays be both sensitive and quantitative and that they be available in a high-density format.

There have been several recent examples of the development and use of protein microarrays (reviewed in refs 1 and 2). Protein arrays have been used to screen the binding specificities of protein expression libraries[3] and for high-throughput screening of antibodies[4,5] and to examine protein–protein,[6–8] protein–DNA, and protein–RNA interactions.[9] Protein microarrays, in an ELISA-format, have also been developed for the measurement of proteins in clinical applications, for instance for the measurement of cytokines in conditioned media and serum,[10–12] prostate-selective antigen (PSA), PSA-ACT and IL-6 in serum,[13] and auto-antibodies in the sera of patients with autoimmune disease.[14]

Protein microarrays for the analysis of clinical samples need to be highly sensitive and quantitative. A variety of different surfaces have been used for making protein microarrays, including membranes, such as nitrocellulose and PVDF,[9,10,14] hydrogels,[15] glass,[6–8,16] and polystyrene.[17] In general, glass slides are the preferred surface for a microarray because of their ease of use, greater durability, optical properties, and the ability to use robotic spotters to generate high-density arrays. While a number of protein microarrays have been developed on glass slides, only a few have been developed for applications requiring high sensitivity. Sensitivities have ranged from 0.1 pg/mL to 1 ng/mL.[6,11,13,14,18] However, the most sensitive microarray developed (0.1 pg/mL), which utilizes the "rolling circle DNA amplification" technology,[18] requires extensive chemical labeling of the detection antibody and is not easily adaptable in other laboratories. Other sensitive assays require specialized equipment[11] or were developed for specific clinical applications such as the diagnosis of autoimmune disease and are not generally applicable.[14] As such, the development of a highly sensitive microarray ELISA that utilizes high-density spotting would advance this technology to a point where it is easily adaptable for high-throughput, quantitative analysis of proteins in clinical or research laboratory settings.

In this paper, we describe a microarray technology that is capable of the sensitive quantitation of hepatocyte growth factor (HGF), a protein recognized as a serum marker for a number of cancers, including breast cancer.[19] By coupling a microarray-ELISA format with the signal amplification of tyramide deposition, we obtain sub-pg/mL sensitivity. We demonstrate the utility of our microarray by comparing the concentration of serum HGF in women with breast cancer and a healthy control group and by showing that our results are comparable to those obtained with a commercial 96-well ELISA. This microarray is simple to prepare and highly sensitive and has the potential to be used to simultaneously analyze large numbers of serum proteins in a rapid and reproducible manner.

Experimental Section

Materials and Reagents. BS^3 and the protein biotinylation kit were from Pierce (Rockford, IL). HGF, HGF-specific, and vascular endothelial growth factor (VEGF)-specific antibodies, as well as the Quantikine ELISA kit for human HGF, were from R&D Systems (Minneapolis, MN). Other antibodies and purified marker proteins include the following: VEGF (Biodesign, Saco,

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ME), CA 15-3 and anti-CA 15-3 antibodies (Fitzgerald, Concord, MA), soluble FAS ligand (Alexis Biochemicals, San Diego, CA), anti-FAS ligand antibodies (BD PharMingen, San Diego, CA), PSA and anti-PSA capture antibody (BiosPacific, Emeryville, CA), biotinylated anti-PSA antibody (Chromaprobe, Aptos, CA). The TSA Biotin System kit including blocking reagent, streptavidin–horseradish peroxidase (HRP) conjugate, biotinyltyramide, and reaction diluent was from Perkin-Elmer (Boston, MA). The Cy3–streptavidin conjugate was from Amersham Pharmacia (Piscataway, NJ). Sera from 10 breast cancer patients and 10 age-matched controls were obtained from the Breast Cancer Serum Biomarkers Resource, Lombardi Cancer Center (Washington, DC). Aminosilanated slides and all chemicals not listed above were obtained from Sigma (St. Louis, MO).

Microarray Preparation. A PixSys 5000 robot from Cartesian Technologies (Irvine, CA) equipped with ChipMaker2 quill pins from TeleChem (Sunnyvale, CA) was used to make the arrays. Aminosilanated slides were modified with 200 μL of a fresh 0.3 mg/mL solution of the homobifunctional cross-linker BS^3 in PBS (Dulbecco's phosphate buffered saline) for 5 min. The slides were rinsed briefly in 70% ethanol and dried under a stream of N_2 gas. An HGF-specific monoclonal "capture" antibody suspended to 1 mg/mL in PBS was printed on the slides. Also printed on each slide were an antibody that does not recognize HGF and a biotinylated protein. The antibody that does not recognize HGF served as a negative control. The biotinylated protein was a positive control for surface attachment and binding of the fluorescent probe (see below). The biotinylated protein also served as a reference when the array was imaged. These proteins were printed as arrays containing five spots of each reagent. Spots were printed either 0.5 or 1 mm apart and were approximately 1 nL in volume. The slides were incubated in a humid chamber for 1 h. Chamber humidity was maintained at 75% during all steps.

HGF Microassay. The arrays were circled with a hydrophobic pen to mark their location and to facilitate probing the array with small volumes. The pen makes a hydrophobic barrier on the surface of the slide, holding the sample in place over the array. During this step, the arrays were permitted to dry for 5–10 min. Each array was then blocked with 50 μL of TNB (100 mM Tris pH 7.5, 150 mM NaCl, 0.5% blocking reagent) for 1 h. The TNB was aspirated from the surface, and each array was incubated overnight with either 50 μL of an HGF standard in TNB or a serum sample diluted 4-fold in TNB (100 μL volumes were used in the high sensitivity experiment). The antigen solution was rinsed off in a gentle stream of water, and the slides were washed three times for 5 min in TNT (100 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween-20). Each array was then probed for 2 h with 50 μL of biotinylated detection antibody diluted in TNB. The biotinyl-anti-HGF antibody was diluted 1:1500 to 67 ng/mL for this step unless noted otherwise. Excess liquid was blotted from the slides, and the slides were washed three times for 5 min with TNT. The TSA–biotin system was then used to amplify the signal. Arrays were incubated for 1 h with 50 μL of streptavidin–HRP conjugate diluted 1:100 in TNB and washed as above. Each array was incubated for 10 min with 50 μL of biotinyltyramide diluted 1:100 in the supplied reaction diluent (or, alternatively, in 100 mM borate pH 8.5, 0.0009% H_2O_2), and the wash procedure was repeated. Each array was probed for 1 h in the dark with 50 μL of Cy3–streptavidin conjugate diluted to 1 μg/mL in TNB. Exposure to the light was avoided while the wash procedure was repeated, and the slides were rinsed twice in water and air-dried. A ScanArray 3000 from General Scanning (Billerica, MA) was used for fluorescence detection of the Cy3. Images thus captured in the ScanArray software were quantitated using ImaGene software (Biodiscovery). For comparison to our microarray ELISA, a commercial 96-well HGF ELISA was performed according to the manufacturer's instructions. Statistical comparison of the HGF levels in breast cancer patients and age-matched controls was undertaken using a **t** test and a probability value of <0.05 with SigmaStat 2.0 software.

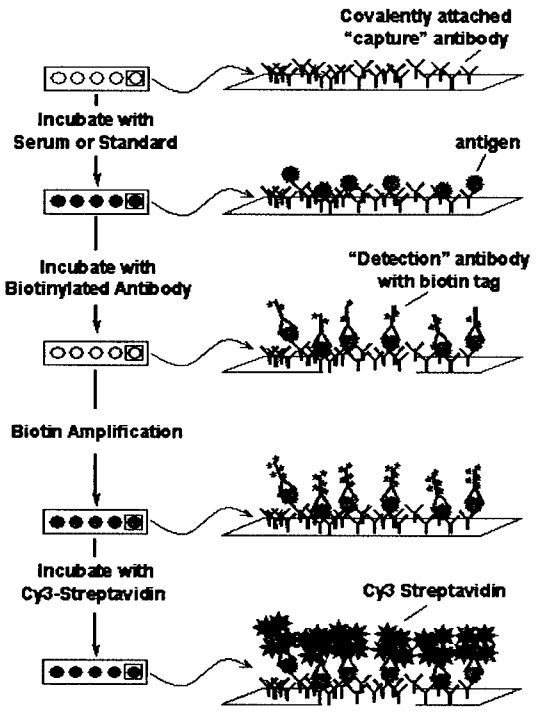

Figure 1. Schematic representation of the microarray "sandwich" ELISA used in this study.

Multiplex Experiment. This experiment was performed essentially as described for the HGF microassay. Capture antibodies for HGF, vascular endothelial growth factor, CA 15–3, FAS ligand, and PSA were spotted as solutions ranging from 0.25 to 1.0 mg/mL. Antigen concentrations were 200 pg/mL HGF, 300 pg/mL VEGF, 30 U/mL (approximately 60 ng/mL) CA 15-3, 200 pg/mL FAS ligand, and 20 pg/mL of PSA. Detection antibodies were used in concentrations ranging from 50 to 500 ng/mL. The CA 15-3 detection antibody was biotinylated using a kit and according to the manufacturer's (Pierce) instructions. All other detection antibodies were purchased as biotin conjugates. Two tyramide amplification steps were performed as described above. The first round of amplification was done after the arrays were exposed to detection antibodies for PSA and FAS ligand only. Subsequently, the arrays were exposed to the remaining detection antibodies and the amplification procedure repeated.

Results and Discussion

The sensitive detection of specific proteins is a major challenge in the development of protein microarrays designed to monitor levels of biomarkers that are often in low abundance. Since proteins cannot be amplified the way nucleic acids can, other methods of signal enhancement must be used if high levels of sensitivity are to be achieved. We have chosen to use an enzymatic signal enhancement method known as tyramide

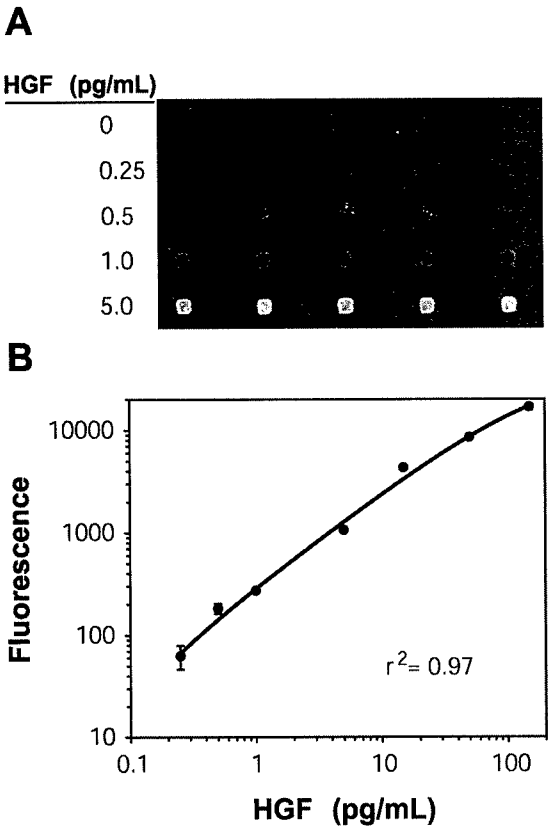

Figure 2. Microassay for HGF is capable of sub-pg/mL sensitivity. (A) The HGF concentration-dependent fluorescent response. Each row of five spots is from a separate array probed with the indicated HGF concentration. Images from separate microarrays are juxtaposed for comparison. (B) A standard curve for the HGF microarray values was calculated using a four-parameter logistic curve. Each data point was weighted by the inverse of the square of fluorescence intensity ($1/y^2$). Each data point represents the mean ± SE of five fluorescent spots for each HGF concentration.

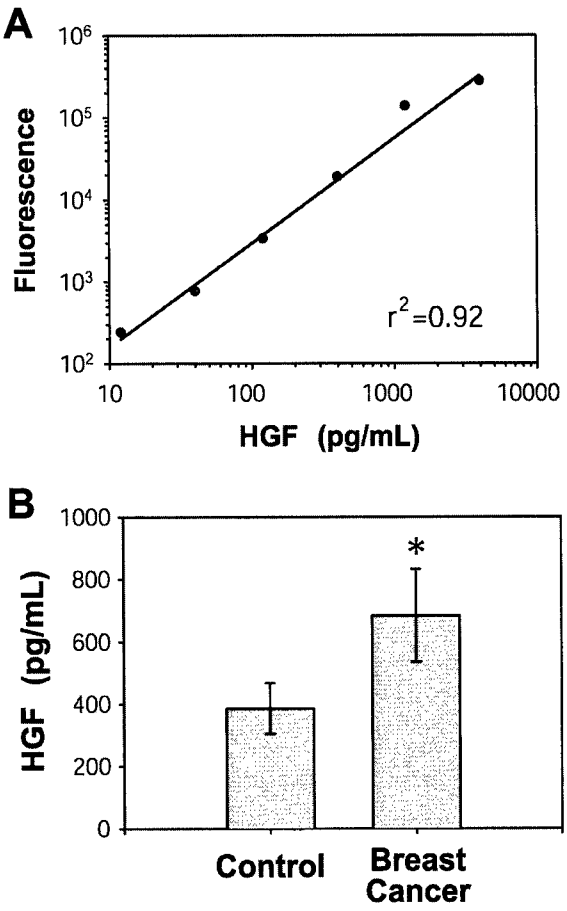

Figure 3. Detection of increased HGF levels in sera from breast cancer patients using the HGF microassay. (A) The HGF standard curve spans over 2.5 orders of magnitude. (B) HGF concentration (mean ± SE) in sera from breast cancer patients (**n** = 10) and normal controls (**n** = 10), as determined using the microassay. *Significantly different from the control group (**P** < 0.05).

signal amplification (TSA). This method has been used extensively in immunohistochemistry, a slide-based protein application, and has been found to provide exceptional sensitivity and low background. It has also been used in quantitative 96-well ELISA formats to detect specific proteins, such as HIV-1 p24 antigen and soluble interleukin 2 receptor, in complex body fluids.[20–22] Therefore, we tested tyramide signal amplification to see if it would be suitable for use with the microarray ELISA analysis.

A schematic diagram of the microarray ELISA approach used in this study is shown in Figure 1. Capture antibodies are covalently attached to a chemically reactive glass slide surface using spot sizes that are compatible with high-density microarrays. These spatially confined antibodies bind a specific antigen from a sample overlaying the array. A second, biotinylated antibody that recognizes the same antigen as the first antibody but at a different epitope is then used for detection. This "sandwich" approach favors specificity in analyte detection, since selective detection is provided sequentially by two separate antibodies. A streptavidin-HRP conjugate is then bound to the biotin moiety of the detection antibody, and catalyzes the TSA reaction. During this reaction the localized deposition of biotin takes place on the surface of all immediately available proteins. Thus the amount of covalently linked biotin in the immediate area is amplified. The biotin is then bound by a Cy3-streptavidin conjugate and the spot quantified using a fluorescence microarray reader. The amplification step does not decrease spot resolution as compared to spots of directly deposited proteins with fluorescent labels (data not shown).

We have successfully employed our microassay in the detection of HGF. By using a 1:200 dilution (0.5 μg/mL) of the detection antibody and 100 μL sample volumes, HGF can be detected down to 0.5 pg/mL (6 fM), equivalent to only 0.6 amol of HGF in the whole sample (Figure 2A). The quantitative range under these conditions approaches 3 orders of magnitude (Figure 2B). As we demonstrate below, we can manipulate the limits of the quantitative range by altering the concentration of the detection antibody. Antibodies that do not recognize HGF were printed as a negative control. The fluorescent intensity at the negative control spots in the presence of even the highest concentrations (1000 pg/mL) of HGF tested was comparable to the intensity of the spots containing anti-HGF capture antibody when incubated in solutions lacking HGF (data not shown). Since the same detection antibody was used in both cases, the low level of background fluorescence is not related to nonspecific binding of the detection antibody or HGF to the spots.

To measure HGF in clinical samples we sought to shift the quantitative range of the assay closer to the physiological range expected for HGF. By further diluting the detection antibody,

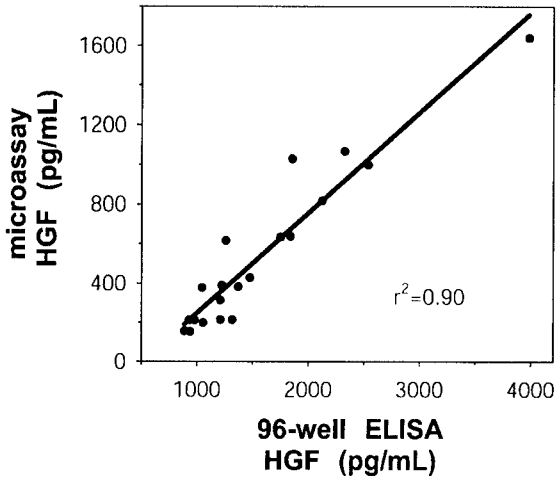

Figure 4. HGF values obtained with the microarray ELISA correlate well with a commercial 96-well ELISA. HGF concentration was measured by both methods in sera from 10 breast cancer patients and 10 age-matched controls.

we obtained a quantitative range from 12 to 4000 pg/mL in the serum (Figure 3A). Since serum samples were diluted 4-fold for this assay, each replicate of the microarray assay used only 12.5 μL of serum. HGF concentrations in clinical samples ranged from 0.15 to 1.64 ng/mL. Sera from 10 breast cancer patients with recurrent disease had a significantly elevated mean HGF concentration of 684 pg/mL (199−1640 pg/mL) compared to 386 pg/mL (153−998 pg/mL) in sera from 10 age-matched normal controls (Figure 3B). This result confirms previous work correlating recurrent breast cancer with higher levels of HGF in serum.[23]

To validate the results obtained with the microassay, we compared the data with that from a commercial 96-well ELISA kit. Data from the two methods showed a linear relationship with a correlation coefficient (r^2) of 0.90 (Figure 4), indicating that both methods produce similar results. Even so, the line describing the relationship between microassay and ELISA data does not have a slope of 1, meaning that the two assays give different absolute values for the HGF concentration in a given sample. It is common for assays based on immunochemical methods to vary in absolute quantitation, and improving their comparability is a recognized challenge.[24] Since the same set of standards but not the same antibodies were used in both assays, differences in results most likely reflect differences in antibody specificities, which may yield variable results due to steric interactions between the antibodies or differential recognition of the antigen due to post-translational modification or partial degradation of the antigen. This point is highlighted by a study in which six different ELISAs reported vastly different

Table 1. Interplate Reproducibility of the Multiplex Microarray ELISA

		signal		
antigen	pg/mL	mean	STD	% CV
HGF	200	2216	207	9.3
VEGF	300	37 831	4775	12.6
CA 15-3	60 000	15 450	1374	8.9
FAS ligand	200	33 092	2591	7.8
PSA	20	23 515	947	4.0

concentrations of tumor necrosis factor in the majority of individual samples.[25] Despite differences between the microassay and the 96-well ELISA, the range of HGF concentrations we found in the sera of breast cancer patients using our microassay (0.199−1.64 ng/mL) is nearly identical to the range found by Maemuro and co-workers (0.15 to 1.43 ng/mL) using a different ELISA kit.[19]

To determine if this technology could be used to simultaneously detect multiple biomarkers, we analyzed five different proteins in a single microarray. The capabilities of the microarray were further tested by analyzing proteins over a wide range of concentrations. The proteins were HGF, VEGF, CA 15-3, FAS ligand, and PSA and were assayed at biologically relevant concentrations[26−32] that ranged from 20 to 60 000 pg/mL (Table 1). Furthermore, we only tested a single antibody pair for each protein. The goal here was to see if it was possible to modify assay conditions to accommodate varying antibody affinities and antigen levels. This approach is more efficient than testing different antibody combinations and may be essential when antibody availability is limited. Initial studies indicated that we could readily detect VEGF and CA 15−3 using incubation and detection conditions similar to those used for HGF, but that FAS ligand and PSA signals were very weak (data not shown). In an effort to increase signal strength, we tried using two tyramide amplification steps for these latter two antigens. In this procedure, the microarray was first incubated with the antigen mixture followed by incubation with biotinylated antibodies to FAS ligand and PSA. The biotin was then amplified using the tyramide deposition procedure. Then the microarray was incubated with a mixture of biotinylated antibodies against the remaining 3 antigens (i.e., HGF, VEGF, and CA 15-3). The subsequent tyramide amplification step would therefore be a second amplification for FAS ligand and PSA but would be the first amplification step for HGF, VEGF, and CA 15-3. Replicate microarray assays were then undertaken using this procedure such that three microarrays were exposed to all five antigens, while individual microarrays were prepared where individual antigens were omitted (Figure 5). Using this approach, we were clearly able to obtain usable signal/background levels for each biomarker (Figure 5). Analysis of

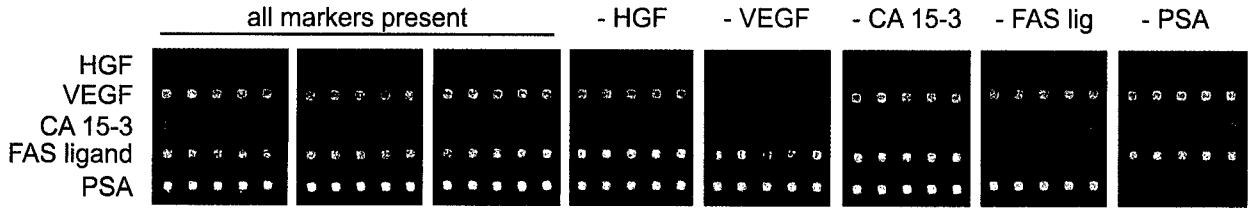

Figure 5. Multiple biomarkers can be simultaneously measured on a single microarray. Eight identical slides were printed with capture antibodies to five different protein markers. Three of these slides were incubated with a mixture of all five antigens (see Table 1), while the other five slides were each incubated with the same mixture of proteins minus a single antigen. Antigens were then detected as described in the text.

the coefficient of variation (CV; the standard deviation divided by the mean) between slides in the multiplex study indicated that the CV values varied from 4 to 12.6% (Table 1). Therefore, these data demonstrate that the microarray ELISA can be easily adapted for the reproducible analysis of multiple antigens, even when the concentrations of the different antigens vary 3000-fold, and there are apparent variations in the quality of the antibodies.

The microassay we describe has many advantageous features, including its small size, sensitivity, and the commercial availability of all reagents and detection equipment. The small size will allow for multiple biomarkers to be analyzed in parallel. That is, with the spot size of ~150 μm that we used, it is possible to make high-density microarrays with 5000−10000 spots per slide. Small size also translates into more efficient use of reagents and precious biological samples such as biopsies or nipple aspirates. Exceptional sensitivity and flexible quantitative range increases the pool of biomarkers that can potentially be assayed, both individually and simultaneously. As such, the ability to vary the quantitative range of individual biomarkers simply by varying the concentration of their respective detection antibodies should prove particularly useful for assaying multiple protein markers on a single microarray. The assay can be prepared and run without the need for customized detection equipment or in-house protein modification, which will facilitate the rapid development and use of similar microarrays in other laboratories.

Conclusions

We developed a protein microarray ELISA suitable for analysis of HGF levels in serum samples. This assay demonstrated exceptional sensitivity and quantitative characteristics comparable with a 96-well ELISA. This technology is readily adaptable for high-throughput, high-density analysis of proteins in clinical and research laboratories.

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